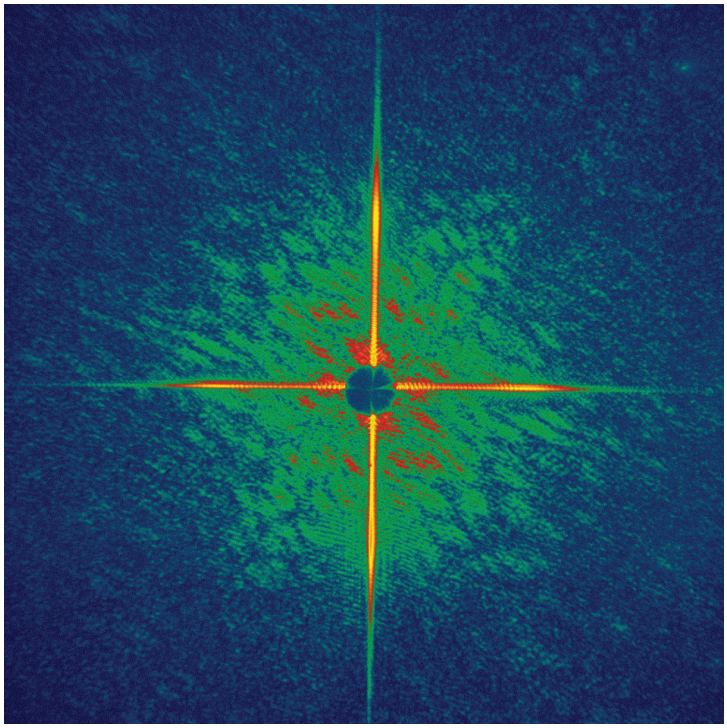
Imaging Complex Biomolecules in a Flash

VIEWING the detailed structure of a virus or protein to determine how that molecule interacts with others can help researchers better understand biological systems. Proteins, for instance, are essential parts of all living organisms. Some of them catalyze biochemical reactions that are vital to metabolism. Others help maintain cell shape or are essential in immune response and cell regeneration. These complex macromolecules can range from 400 to about 27,000 amino acids in length, and their structures are a three-dimensional (3D) tangle of precise folds and twists. Furthermore, proteins may shift between several related structures during their normal biological functions. Determining a protein's 3D structure provides important clues about its behavior and function.

Scientists usually recover 3D images of large macromolecules with x-ray crystallography, which uses x rays to bombard a crystal. As x-ray photons pass through the closely spaced lattice of atoms forming the crystal, some of them are diffracted. Recording this diffraction pattern can reveal information about the crystal lattice and its constituents, which helps researchers determine a material's molecular structure.

Unfortunately, with this technique, the targeted material must be in crystalline form, and not all biological macromolecules can be crystallized. For example, many drugs developed to treat diseases target proteins that bind to membranes, but these proteins resist crystallization. Having more detailed information about protein structures and behavior could significantly benefit treatment options and improve human health.

A team of Livermore researchers in collaboration with colleagues from the University of California at Davis, Stanford Synchrotron Radiation Laboratory, Uppsala University in Sweden, and the Deutsches Elektronen-Synchrotron (DESY) in Germany is working to solve this problem. Led by physicist Henry Chapman in Livermore's Physics and Advanced Technologies Directorate, the team is developing a method that will use the extremely bright x rays generated by the Linac Coherent Light Source (LCLS) at the Stanford Linear Accelerator Center to examine the hidden structural details of biological molecules.



An experiment with a prototype x-ray free-electron laser at the Deutsches Elektronen-Synchrotron in Germany produced this initial diffraction pattern.

Blasted to Bits

In the late 1980s, Livermore scientists demonstrated that x-ray lasers could be adapted for biological imaging. Those experiments used an extremely short x-ray pulse generated by a Nova laser beam and a diffractive optical x-ray lens to form images of cells. However, the pulses were not bright enough to produce high-resolution images of macromolecules. In addition, the lenses were not suitable for this application.

LCLS will solve this challenge when it comes on line in 2009. The world's first x-ray free-electron laser (FEL), LCLS will emit x rays in the form of a laser beam with a brightness 10 billion times greater than existing x-ray sources. Its 0.15-nanometer wavelength pulses, which are about 100 femtoseconds long, will provide the beam qualities needed to image single macromolecules at the atomic scale. Chapman is working with Janos Hajdu, a professor at Uppsala University and SLAC, to determine the optical requirements for the proposed experiments. Hajdu was one of the first to propose using such pulses for biomolecular imaging.

Another challenge for the Livermore team was to capture the faint patterns scattered from the sample before the macromolecule

or particle explodes—a time span of about 10 to 50 femtoseconds (where 1 femtosecond equals 10^{-15} seconds). To solve this problem, the Livermore team combined a graded, multilayer mirror with a charge-coupled device detector. "We have a signal-to-noise problem," says Chapman. "We must be able to detect a single photon scattering from a beam of a trillion photons." The multilayer mirror reflects the single photons of interest and filters out the unrelated photons and plasma radiation.

In designing the detector, the team applied the Laboratory's expertise in extreme-ultraviolet lithography. "Livermore has an exceptional capability for making complex multilayer mirrors," says Chapman. "The mirrors must allow scattered photons of a particular wavelength to pass through and block the others."

The detector must quickly record the diffraction pattern, before the focused x-ray pulse turns the sample into plasma. No x-ray experiments had been conducted in the relevant time and intensity domains, so the Livermore team used computer simulations to evaluate experimental setups. Modeling results indicated that nearly atomic-level resolution could be obtained by carefully choosing the pulse length, intensity, and x-ray wavelength.

FLASH First, Then Shrinkwrap

The team's initial experiments of the diffractive imaging technique used FLASH, a prototype soft-x-ray FEL developed at DESY. In these demonstration experiments, FLASH produced coherent FEL pulses, each lasting 25 femtoseconds and containing about a trillion (10^{12}) photons. Targets included latex spheres 100 nanometers in diameter and 20-nanometer-thick silicon nitride membranes with patterns cut through them by a focused ion beam. Experiments on both sample types were conducted in a vacuum chamber. The x-ray beam illuminated and passed through the sample and exited through a hole in the graded, multilayer planar mirror. Light scattered by the sample reflected off the mirror onto a charge-coupled device, which recorded the diffraction pattern.

The information encoded in this pattern is similar to that from a hologram and must be transformed into an image of the original sample. "To convert the signal data into an image, we must determine the phase of the scattered waves, but we can only measure the intensity of those waves," says Chapman. "We correlate intensity to the strength of the spatial frequencies in an image, but we still need the phases to properly sum the frequencies and form the image."

One diffraction pattern consists of more than a million data points, which translates to more than a million unknown phases that must be determined. However, because many configurations will yield the same pattern, researchers needed a method to determine which one is correct.

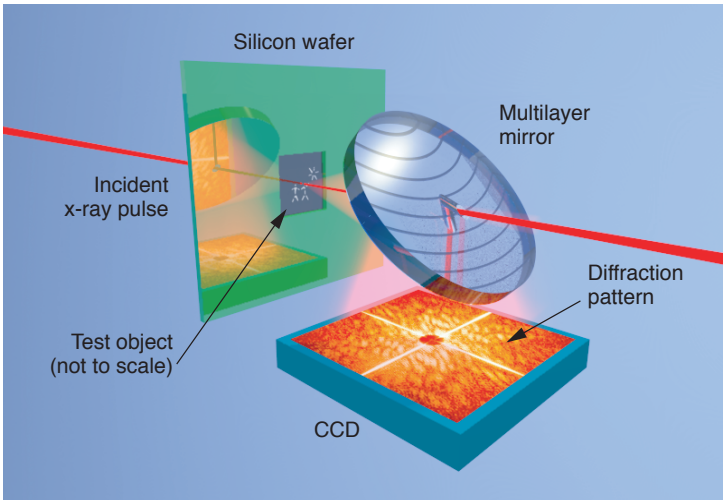
To solve this problem, the team used Shrinkwrap, an iterative transform algorithm based on methods used for astronomical imaging. Developed at Livermore by Stefano Marchesini, Shrinkwrap recovers an image in an iterative process, starting from some simple constraints. "Basically, we assume that the object is isolated with nothing around it of interest," says Chapman. "All spatial frequencies must then cancel outside the object's boundaries, adding up to zero."

Shrinkwrap iterates back and forth between the image and its calculated diffraction pattern, imposing the constraints in both spaces. Initially, the algorithm chooses a loose estimate because the object's boundary is unknown. As the iterations proceed, Shrinkwrap refines the boundary constraint and conforms to the object's shape. "The tighter the algorithm wraps around the object, the better it estimates the details and thus the boundary constraint," Chapman says. "Plus it does not require previous knowledge of an object's boundaries."

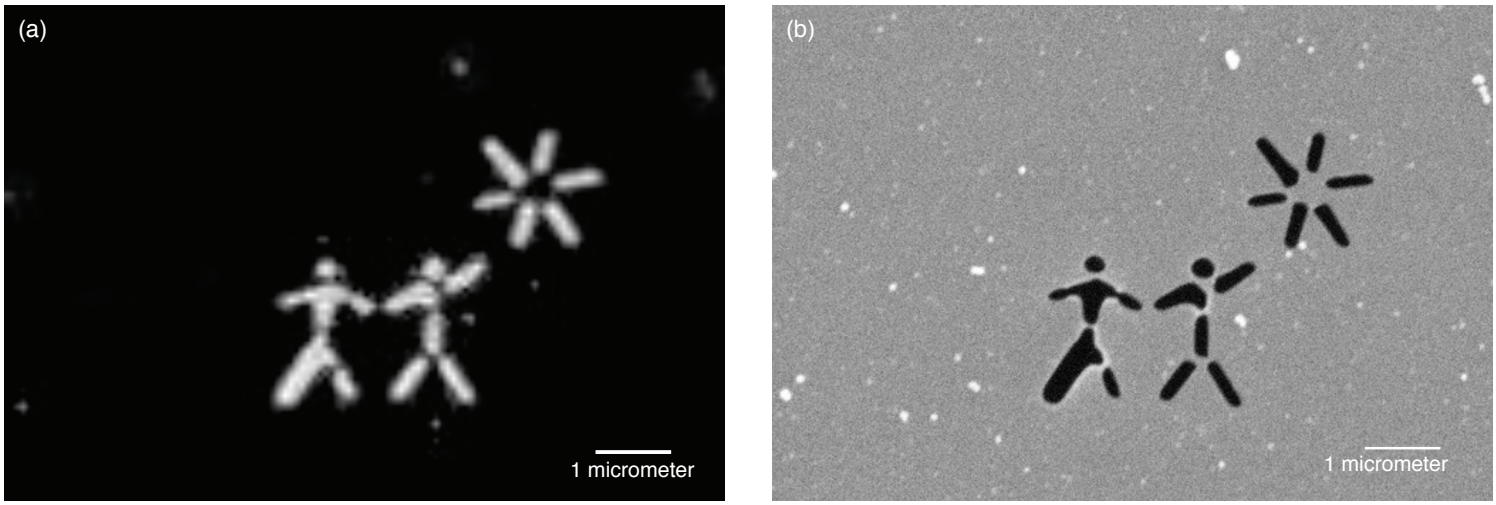
To test the technique, the team carried out many reconstructions, each time starting from random phases. Because of photon noise in the data, no solution can exactly satisfy all of the constraint sets. However, demonstration tests showed that by the final iteration, each image is clearly recognizable when compared with the micrograph taken of the object.

Seeing into the Future

This research, which was funded by Livermore's Laboratory Directed Research and Development Program, resulted in the first diffraction patterns to be created with an ultrafast pulse of soft x rays and transformed back into the original image. Featured on the December 2006 cover of *Nature Physics*, the images are thought to be the fastest ever formed. Chapman and his colleagues



In a diffractive imaging experiment using an x-ray free-electron laser, the x-ray beam is focused to a spot 20 micrometers in diameter. A multilayer mirror reflects the diffracting x rays onto a charge-coupled device (CCD). The powerful direct beam passes through a hole in the mirror to a beam dump.



The Shrinkwrap algorithm transformed the diffraction pattern shown on p. 21 into (a) an image of the targeted object, which can be compared to (b) a micrograph of the target. The pulse from the free-electron laser destroyed the object, but the destruction took longer than the pulse's 25-femtosecond duration.

are looking forward to testing the technique on LCLS, which will provide images with even higher resolution. Meanwhile, they continue to refine the process.

In March 2007, they injected submicrometer-size latex beads and biological cells into the beam using a technology developed at Livermore for use in the bioaerosol mass spectrometry system. (See *S&TR*, September 2003, pp. 21–23.) In addition, the FLASH machine is being upgraded at DESY so that it will produce even shorter pulses and wavelengths, which will reveal greater details at smaller dimensions. With LCLS and other hard-x-ray systems on the horizon, the time is coming when proteins, viruses, and complex biological macromolecules will give up their structural secrets, right down to the atomic scale.

—Ann Parker

Key Words: biological macromolecule, diffraction pattern, FLASH, Linac Coherent Light Source (LCLS), protein, Shrinkwrap algorithm, virus, x-ray free-electron laser (FEL).

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